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57th Search

FILE 'HOME' ENTERED AT 10:19:58 ON 02 JAN 2004

L1 32 (SGT AND TRYPSIN OR STEPTOMYCES (A) GRISEUS (A) TRYPSIN OR TRYPS  
IN (S) PRONASE) AND (ANGININE OR L-ARGININE OR BENZAMIDINE)

(FILE 'HOME' ENTERED AT 10:19:58 ON 02 JAN 2004)

FILE 'MEDLINE, CAPLUS, BIOSIS, EMBASE, SCISEARCH' ENTERED AT 10:20:27 ON  
02 JAN 2004

L1 32 S (SGT AND TRYPSIN OR STEPTOMYCES (A) GRISEUS (A) TRYPSIN OR TR  
L2 18 DUP REM L1 (14 DUPLICATES REMOVED)  
L3 1 S L2 AND (L-ARGININE OR ARGININE) AND BENZAMIDINE  
L4 16 S L2 NOT PY>2001

L4 ANSWER 3 OF 16 MEDLINE on STN  
AN 81117194 MEDLINE  
DN 81117194 PubMed ID: 7462203  
TI Interactions of derivatives of guanidinophenylglycine and  
guanidinophenylalanine with trypsin and related enzymes.  
AU Tsunematsu H; Makisumi S  
SO JOURNAL OF BIOCHEMISTRY, (1980 Dec) 88 (6) 1773-83.  
Journal code: 0376600. ISSN: 0021-924X.  
CY Japan  
DT Journal; Article; (JOURNAL ARTICLE)  
LA English  
FS Priority Journals  
EM 198104  
ED Entered STN: 19900316  
Last Updated on STN: 19970203  
Entered Medline: 19810424  
AB Ethyl N-benzoyl-p- and m-guanidino-DL-phenylglycinates (DL-Bz-p-GPG-OEt  
and DL-Bz-m-GPG-OEt), and ethyl N-benzoyl-p-guanidino-L- and  
D-phenylalaninates (L-Bz-p-GPA-OEt and D-Bz-p-GPA-OEt) were synthesized.  
The ester of the racemic p-guanidinophenylglycine derivative was  
completely hydrolyzed by **trypsin**, **pronase**,  
alpha-chymotrypsin, and thrombin, though hydrolysis by the latter two  
enzymes was much slower. Papain hydrolyzed this ester substrate  
stereospecifically at a moderate rate and left the ester derivative of the  
D-enantiomer unaltered. Optical resolution of DL-Bz-p-GPG-OEt with papain  
gave N-benzoyl-p-guanidino-L-phenylglycine (L-Bz-p-GPG-OH) and the ester  
of the D-enantiomer of this amino acid derivative. On the other hand,  
DL-Bz-m-GPG-OEt was completely hydrolyzed by **pronase** and was  
stereospecifically hydrolyzed by papain, but was unaffected by  
**trypsin**, alpha-chymotrypsin, and thrombin. The trypsin-catalyzed  
hydrolysis of N alpha-benzoyl-L-**arginine**  
p-nitroanilide (L-Bz-Arg-pNA) was inhibited competitively by this ester.  
The specificity constant (kcat/Km) for L-Bz-p-GPG-OEt was about 57 times  
smaller than that for a specific ester substrate, ethyl N  
alpha-benzoyl-L-argininate (LO-Bz-Arg-OEt), while the value for the  
D-enantiomer of the former is about 14 times larger than that for the  
D-enantiomer of the latter. L-Bz-p-GPA-OEt has a specificity constant  
comparable to that for L-Bz-Arg-OEt. The value for the former is about 51  
times larger than that for L-Bz-p-GPG-OEt. This suggests that the  
existence of the beta-methylene group in L-Bz-p-GPA-OEt is important in  
relation to the higher susceptibility of the ester to trypsin-catalyzed  
hydrolysis. In contrast with the L-enantiomer, D-Bz-p-GPA-OEt was found  
to be as competitive inhibitor for the hydrolysis of L-Bz-Arg-pNA. A  
significant difference was found between the stereospecificities of  
hydrolysis of the ester substrates of the two amino acid derivatives by  
trypsin.

L4 ANSWER 4 OF 16 MEDLINE on STN  
AN 76069297 MEDLINE  
DN 76069297 PubMed ID: 399  
TI Proteolytic enzymes of the K-1 strain of Streptomyces griseus obtained  
from a commercial preparation (Pronase). Purification and characterization  
of the carboxypeptidase.  
AU Seber J F; Toomey T P; Powell J T; Brew K; Awad W M Jr  
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1976 Jan 10) 251 (1) 204-8.  
Journal code: 2985121R. ISSN: 0021-9258.  
CY United States  
DT Journal; Article; (JOURNAL ARTICLE)  
LA English  
FS Priority Journals

EM 197603  
ED Entered STN: 19900313  
Last Updated on STN: 19970203  
Entered Medline: 19760301  
AB We described earlier the facilitated purifications of the **trypsin** and aminopeptidase components present in **Pronase** (Vosbeck, K. D., Chow, K. -F., and Awad, W. M., Jr. (1973) J. Biol. Chem. 248, 6029-6034). A partially resolved protein mixture left over after one of the steps in that procedure was passed through a Sephadex G-75 column. By this means, a component with carboxypeptidase activity was separated from associated serine endopeptidases. Further purification of this exopeptidase to apparent homogeneity was achieved by refiltration through the same Sephadex column and by CM-cellulose chromatography. A single protein band was observed after acrylamide gel electrophoresis; analysis by sedimentation equilibrium using the meniscus depletion method gave a molecular weight of 30,300. This enzyme demonstrates activity against Nalpha-benzoyloxycarbonylglycyl-L-leucine and hippuryl-D,L-phenyllactate; no activity was found against Nalpha-acetyl-L-tyrosine ethyl ester, Nalpha-benzoyl-D,L-**arginine**-p-nitroanilide, or L-leucine-p-nitroanilide. The maximum activity lies between pH values of 7 and 8; the enzyme is stable between pH values of 6 and 10. At room temperature 1,10-phenanthroline inactivates the enzyme completely whereas EDTA has no effect. Of the many cations tested, only Co<sup>2+</sup>, Ni<sup>2+</sup>, or Zn<sup>2+</sup> restores activity to the 1,10-phenanthroline-treated enzyme; Co<sup>2+</sup> provided 3 times the native activity. The metal in the native protein was found to be zinc. These findings are similar to those recorded with bovine pancreatic carboxypeptidase A, and suggest the possibility that the present enzyme may be genetically related to the mammalian protein, as in previously noted examples of homology of three Pronase endopeptidases to pancreatic serine enzymes.

L4 ANSWER 5 OF 16 MEDLINE on STN  
AN 75127939 MEDLINE  
DN 75127939 PubMed ID: 235280  
TI Enzymic and physicochemical properties of Streptomyces griseus trypsin.  
AU Olafson R W; Smillie L B  
SO BIOCHEMISTRY, (1975 Mar 25) 14 (6) 1161-7.  
Journal code: 0370623. ISSN: 0006-2960.  
CY United States  
DT Journal; Article; (JOURNAL ARTICLE)  
LA English  
FS Priority Journals  
EM 197507  
ED Entered STN: 19900310  
Last Updated on STN: 19950206  
Entered Medline: 19750707  
AB Streptomyces griseus **trypsin** has been isolated from **Pronase** by ion-exchange chromatography on CM-Sephadex and SE-Sephadex. The isolated enzyme was homogeneous by the criteria tested except for a low degree of contamination by an enzyme with nontryptic activity. The latter could be partially resolved by chromatography on Bio-Rex 70. The molar absorptivity at 280 nm was found to be 3.96 times 10<sup>-4</sup> M<sup>-1</sup>/cm and the E1cm1% was found to be 17.3. The molecular weight was 22,800 plus or minus 800. The enzyme was found to be stable at 0 degrees from pH 2 to 10. At 30 degrees the enzyme was maximally stable at pH 3-4 and significantly stabilized in the neutral and alkaline range by 15 mM Ca<sup>2+</sup>. Some evidence was obtained for a reversible denaturation of the enzyme at pH 12.0 and 2.0. The K<sub>m</sub> for N-alpha-benzoyl-L-**arginine** ethyl ester at pH 8.0 in 20 mM CaCl<sub>2</sub>-0.1 M KCl-10 mM Tris-HCl buffer at 30 degrees was found to be 7.7 plus or minus 1.9 times 10<sup>-6</sup> M and the esterase activity was observed to be dependent on an

ionizing group with pK-a equals 5.85. In 2H<sub>2</sub>O this pK<sub>a</sub> was increased to 6.35 and the rate of hydrolysis decreased threefold. The rate of hydrolysis was independent of pH between 8 and 10. The inhibition of the enzyme with L-1-chloro-3-tosylamido-4-phenyl-2-butanone was shown to be associated with the alkylation of its single histidine residue. This residue is present in a homologous amino acid sequence as the active-site histidine in trypsin and chymotrypsin. Optical rotatory dispersion and circular dichroism measurements over the pH range 5.3-10.5 indicated no significant conformational change until the pH was increased above 10.1. The observation that, under the conditions tested, acetylation and carbamylation of the NH<sub>2</sub>-terminal valine were incomplete is consistent with the view that this group is buried as an ion pair and only becomes available for deprotonation and reaction upon denaturation of the enzyme at pH values greater than 10.0.

L4 ANSWER 10 OF 16 CAPLUS COPYRIGHT 2004 ACS on STN

AN 1975:1569 CAPLUS

DN 82:1569

TI Proteolytic enzymes of the K-1 strain of *Streptomyces griseus* obtained from a commercial preparation (**pronase**). VI. Stabilization of the **trypsin** component by calcium and guanidine

AU Russin, David J.; Floyd, Benjamin F.; Toomey, Thomas P.; Brady, Al H.; Awad, William M., Jr.

CS Sch. Med., Univ. Miami, Miami, FL, USA

SO Journal of Biological Chemistry (1974), 249(19), 6144-8

CODEN: JBCHA3; ISSN: 0021-9258

DT Journal

LA English

AB *Streptomyces griseus* **trypsin** was more thermolabile than the 2 other components in **pronase** which was homologous with bovine chymotrypsin. It was completely inactivated after heating to 60.degree. for 15 min. The heat stability of the enzyme was reduced in the presence of EDTA. Ca was the specific cation which stabilized the enzyme at higher temps. This trypsin denatured irreversibly in 8M urea (at 23.degree.) in low Ca<sup>2+</sup> concn., but was stable and active in this denaturant if 0.5M Ca<sup>2+</sup> was present. This latter property makes this enzyme a possibly useful agent in protein structural studies. Both the microbial and bovine trypsin bound guanidinium ion substantially. Guanidinium ion competitively inhibited the activity of each enzyme against N.alpha.-benzoyl-L-arginine-p-nitroanilide (I). Microbial trypsin had about 3-fold greater affinity for guanidine and about a 20-fold lower K<sub>m</sub> for I than did bovine, trypsin. Binding of guanidine with either enzyme produced no apparent inhibition of activity against the poor nonspecific substrate, p-nitrophenyl acetate, when compared to inhibitor-free solns. These findings suggest that guanidine assoc. with that part of the specificity site which binds the charged portion of basic substrate residues. The addn. of 0.2M guanidine-HCl to an 8M urea-10mM CaCl<sub>2</sub> soln. completely inhibited the autolysis of the microbial trypsin but only slightly decreased the rate of autolysis of the bovine enzyme. In 8M urea-10mM CaCl<sub>2</sub> and 1.0M guanidine-HCl, .apprx.90% of the activity of the microbial enzyme was retained after 2 hr even in the presence of another *S. griseus* serine endopeptidase known to be active and stable in this mixed denaturant soln. Therefore, guanidine appears to stabilize microbial trypsin. In the presence of Na EDTA and denaturant mixt., the microbial enzyme rapidly lost activity. Measurements of CD were made at pH 8 and revealed that 0.45M CaCl<sub>2</sub> completely protected the microbial enzyme against rapid unfolding by 8M urea, whereas this salt had little effect upon the rapid conformational transition of the bovine enzyme in this denaturant. Also 0.2M guanidine with a low Ca<sup>2+</sup> concn. could largely stabilize in 8M urea the conformation of the microbial enzyme. A study was carried out to see if the guanidine complex of either

trypsin could demonstrate a changed specificity toward N-acylaminoacyl-p-nitroanilides. The results were entirely neg.

L4 ANSWER 11 OF 16 CAPLUS COPYRIGHT 2004 ACS on STN  
AN 1974:565532 CAPLUS  
DN 81:165532  
TI Proteolytic enzymes of the K-1 strain of Streptomyces griseus obtained from a commercial preparation (**pronase**). VII. Acetylation of **pronase trypsin**  
AU Awad, William M., Jr.; Ochoa, Maria S.  
CS Sch. Med., Univ. Miami, Miami, FL, USA  
SO Biochemical and Biophysical Research Communications (1974), 59(2), 527-34  
CODEN: BBRCA9; ISSN: 0006-291X  
DT Journal  
LA English  
AB Reaction of **Pronase trypsin** with Ac2O yielded a homogeneous, active, and stable deriv. This was achieved by including glycerol in the acetylation reaction as previously described (Siegel, S., Awad, W. M., Jr., 1973). Acetylation resulted in no change in Km and only a moderate decrease in Vmax with N.alpha.-benzoyl-L-**arginine**-p-nitroanilide as substrate. As with bovine trypsin the single N-terminal residue was not acetylated. This is in contrast to the other homologous mammalian and microbial enzymes where complete acetylation of N-terminal residues are noted. Thus, a close conformational homology is suggested around the N-termini of the microbial and mammalian trypsins.